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Health and Environmental Effects Profile for Direct Brown 95

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR DIRECT BROWN 95

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16. ABSTRACT

The Health and Environmental Effects Profile for Direct Brown 95 was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. Direct Brown 95 has been evaluated as a carcinogen. The human carcinogen potency factor (q_1^*) for Direct Brown 95 is 9.3 $(mg/kg/day)^{-1}$ for oral exposure.

The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified by CERCLA based on chronic toxicity. The RQ value for Direct Brown 95 is 100.

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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 104 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1985.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Reference doses (RfDs) for chronic exposures. An RfD is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, RfDs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA and CERCLA dockets.

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The initial draft of this report was prepared by Syracuse Research Corporation under Contract No. 68-03-3220 for U.S. EPA's Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH. Dr. Christopher DeRosa was the Technical Project Monitor and Peter Wallace was the Project Officer. The final documents in this series were prepared for the Office of Solid Waste, Washington, DC.

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EXECUTIVE SUMMARY

Direct Brown 95 is the common name for a commercial azo dye that is produced by the coupling of benzidine, salicylic acid, cresotic (phenol-sulfonic) acid and resorcinol. The product is treated with a copper salt to form a copper complex. This dye is soluble in water with the production of an orange-brown solution and is slightly soluble in ethanol but insoluble in acetone (Society of Dyers and Colourists, 1971). It can be used in the dyeing, staining and printing of cellulose, silk, leather, paper, wool, acetate, nylon and casein-formaldehyde plastics (IARC, 1982a). Four U.S. manufacturers produced 595 thousand pounds of this dye in 1976 (USITC, 1977), but only one U.S. manufacturer reported production of Direct Brown 95 in 1984 (USITC, 1985). Two U.S. companies currently market Direct Brown 95 under the tradenames Direct Brown BRL NB and Elcomine Brown BRL (AATCC, 1985).

Very little experimental data specific to Direct Brown 95 are available to predict its environmental fate. Porter (1973) concluded that Direct Brown 95 is relatively stable to direct photolysis in natural waters. Biodegradation studies with Direct Brown 95 have indicated relatively poor biodegradation based on 5- and 20-day BOD (Atlantic Chemical Co., 1982). If released to water, Direct Brown 95 may be susceptible to significant adsorption because dyestuffs by their substantive nature (ability to be exhaustively deposited to fibers from aqueous baths) are likely to be adsorbed onto both sewage works sludge and silts and sediments of rivers and lakes (Brown and Laboureur, 1983). Removal of adsorbed Direct Brown 95 may occur by anaerobic biodegradation, since a number of azo dyes have been found to be substantially biodegraded under anaerobic conditions (Brown and

(Lebourau, 1983). Hydrolysis, volatilization and bioconcentration are not expected to be significant; adsorption and microbial degradation are expected to be the important fate processes in water. If released to the atmosphere, Direct Brown 95 will likely be associated with particulate matter and exist mainly as a vapor/particulate partition. If released to soil, Direct Brown 95 may not be subject to significant leaching since dyestuffs are susceptible to significant adsorption. Reductive degradation within soil may occur by anaerobic microbial processes (Yoshida et al., 1971b). Photodegradation beyond soil surfaces is not expected to be important.

Occupational exposure to Direct Brown 95 occurs during production and use in dyeing (IARC, 1982a). Wastewater effluents are probably the major source of release to the aquatic environment. Ambient monitoring data could not be located in the available literature as cited in the Appendix.

Little et al. (1974) reported the 96-hour LC_{50} of Direct Brown 95 to fathead minnows, Pimephales promelas, to be >180 mg/L. This value is substantially higher than the concentrations that produce color (0.4-1.1 mg/L) (Little et al., 1974). Other data concerning the aquatic toxicity of this compound could not be located in the available literature as cited in the Appendix.

Pertinent data regarding the absorption and distribution of Direct Brown 95 could not be located in the available literature as cited in the Appendix. Walker (1970) suggested that benzidine-based dyes may be degraded in the gut and liver to free benzidine and monoacetylbenzidine. Benzidine and monoacetylbenzidine were detected in the urine of workers occupationally exposed to benzidine-based dyes (NIOSH, 1980a), and in the urine of rats, mice and monkeys orally-administered Direct Brown 95 (NCI, 1978; Rinde and Troll, 1975).

ale rats, inducing liver tumors

ion (NCI, 1978). No neoplastic

:e of either sex in the 13-week

epidemiological studies (Yoshida

ssociating occupational exposure

and the finding of benzidine in

NIOSH, 1980a) were sufficient to

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,d). The data were inadequate,

occupational exposure to Direct

agenic to S. typhimurium in the

either by a reducing agent or

et al., 1984; Joachim et al.,

hepatocytes when administered in

1984). Pertinent data regarding

not available.

978), dose-related decreased body

and at ≥6000 ppm in mice. Dose-

rats at ≥750 ppm. Nonneoplastic

hanges, lymphoid depletion of the

bone marrow, biliary hyperplasia

ects were observed in rats at 190

at ≥350 ppm (lowest dose tested),

om, hemosiderosis of the kidney at

t 12,500 ppm.

A q_1^* of 9.3 $(mg/kg/day)^{-1}$ was derived based on the dose-response data for liver tumors in female rats fed Direct Brown 95 in the diet for 13 weeks in the NCI (1978) study. The water concentrations associated with increased lifetime risk at levels of 10^{-5}, 10^{-6} and 10^{-7} are 3.8×10^{-5}, 3.8×10^{-6} and 3.8×10^{-7} mg/ℓ, respectively, assuming a 70 kg man drinks 2 ℓ water/day. An F factor of 103 $(mg/kg/day)^{-1}$ was also calculated from these data; the magnitude of F places the dye in Potency Group 1. Evidence that Direct Brown 95 is carcinogenic in animals is limited and in humans is inadequate, but occupational exposure to benzidine-based dyes, in general, is strongly associated with bladder cancer; therefore, Direct Brown 95 was placed in U.S. EPA Group A. A U.S. EPA Group A, Potency Group 1 chemical has a high hazard ranking under CERCLA.

An RQ of 100 was derived based on the increased mortality of male rats in the NCI (1978) study.

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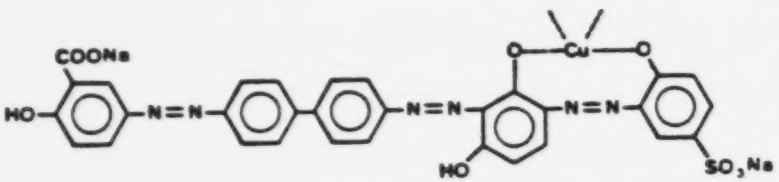
LIST OF ABBREVIATIONS

BOD	Biochemical oxygen demand
CAS	Chemical Abstract Service
COD	Chemical oxygen demand
CS	Composite score
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
FMN	Flavin mononucleotide
HPLC	High pressure liquid chromatography
K_{ow}	Octanol/water partition coefficient
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
MED	Minimum effective dose
MTD	Maximum tolerated dose
ppb	Parts per billion
ppm	Parts per million
RfD	Reference dose
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value
UDS	Unscheduled DNA synthesis

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Direct Brown 95 is a common name for the compound disodium (5-[(4'-((2,6-dihydroxy-3-((2-hydroxy-5-sulfophenyl)azo)phenyl)azo-4-biphenyl)azo]-salicylato (4))-cyprate(2-) according to the IUPAC System. The current CAS designation for Direct Brown 95 is cuprate(2-), (5-[(4'-((2,6-dihydroxy-3-((2-hydroxy-5-sulfophenyl)azo)-phenyl)azo)(1,1'-biphenyl)-4-yl)azo]-2-hydroxy-benzato(4-)), disodium salt. Direct Brown 95 is currently marketed in the United States under the tradenames Direct Brown BRL NB (Fabricolor Inc.) and Elcomine Brown BRL (International Dyestuffs Corp.) (AATCC, 1985). The structure, molecular weight, empirical formula and CAS number are as follows:



Molecular weight: 760.1

Empirical formula: $C_{31}H_{18}CuN_6Na_2O_9S$

CAS Registry No.: 16071-86-6

1.2. PHYSICAL AND CHEMICAL PROPERTIES

Direct Brown 95 is soluble in water (yielding an orange brown solution), slightly soluble in ethanol and insoluble in acetone (Society of Dyers and Colourists, 1971). All direct dyes are water soluble and form anions by dissociation (Kuehni et al., 1979). In aqueous solution, the molecules of direct dyestuffs are linked by hydrogen bonds forming larger agglomerates or colloidal solutions, which reduces their solubility and promotes disposition on cellulosic fibers. The structure of these agglomerates allows firm linkage to the cellulose molecule by multiple hydrogen bonds (Kuehni et al., 1979).

Physical properties such as melting point, boiling point, vapor pressure, density and log K_{ow} could not be located in the available literature as cited in the Appendix. Computer calculation of log K_{ow} was not possible because of the lack of appropriate fragment values (U.S. EPA, 1986a). The air conversion factor for Direct Brown 95 is 1 ppm = 31.6 mg/m^3.

The manufacture and testing of Direct Brown 95 do not conform to rigid chemical specifications and its composition may vary in order to meet shade and intensity requirements (IARC, 1982a). The residual benzidine content of the Direct Brown 95 marketed by Fabricolor Inc., has been reported to be 19 ppm (Jones, 1979).

1.3. PRODUCTION DATA

Direct Brown was first synthesized in 1931 (IARC, 1982a). It is prepared by 1) coupling diazotized benzidine with salicylic acid; 2) coupling this intermediate with 2-amino-1-phenol-4-sulfonic acid previously coupled to resorcinol; and 3) forming a copper complex of the product with a copper salt (Society of Dyers and Colourists. 1971).

In 1976, four U.S. companies produced 595 thousand pounds of Direct Brown 95 (USITC, 1977), which is the most recent production figure available. Crompton and Knowles Corp. is the only U.S. manufacturer listed for this dye in 1984 (USITC, 1985). Direct Brown 95 is commercially marketed under tradenames by Fabricolor Inc. and International Dyestuffs Corp. (see Section 1.1.). The production data available from the public portion of the TSCA Production File for 1977 are listed in Table 1-1.

TABLE 1-1

Direct Brown 95 Production Data for 1977*

Producer, Location	Manufacturer/ Importer	Production Range (pounds)
Fabricolor Inc., Patterson, NJ	manufacturer	0.1-1.0 million
GAF Corporation, Rensselaer, NY	manufacturer	0.1-1.0 million
Ugine Kuhlmann of America, Paramus, NJ	importer	confidential
Pfister Chemical, Ridgefield, NJ	importer	1-10 thousand

*Source: U.S. EPA, 1977

Import of Direct Brown 95 through principal U.S. customs districts in recent years has been reported as follows (USITC, 1981, 1982, 1984):

Year	Import Volume (pounds)
1983	27,281
1981	20,986
1980	24,065

1.4. USE DATA

Direct Brown 95 can be used to dye cellulose, silk fibers, leather, paper and casein-formaldehyde plastics; to stain wool, acetate and nylon fibers; print cellulose and silk fabrics; and to produce heavy metal salts that can be used as pigments (IARC, 1982a).

1.5. SUMMARY

Direct Brown 95 is the common name for a commercial azo dye that is produced by the coupling of benzidine, salicylic acid, 2-amino-1-phenol-4-sulfonic acid and resorcinol. The product is treated with a copper salt to form a copper complex. This dye is soluble in water with the formation of an orange-brown solution and is slightly soluble in ethanol but insoluble in acetone (Society of Dyers and Colourists, 1971). It can be used in the dyeing, staining and printing of cellulose, silk, leather, paper, wool, acetate, nylon and casein-formaldehyde plastics (IARC, 1982a). Four U.S. manufacturers produced 595 thousand pounds of this dye in 1976 (USITC, 1977), but only one U.S. manufacturer reported production of Direct Brown 95 in 1984 (USITC, 1985). Two U.S. companies currently market Direct Brown 95 under the tradenames Direct Brown BRL NB and Elcomine Brown BRL (AATCC, 1985).

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. WATER

2.1.1. Hydrolysis. Experimental hydrolysis data could not be located in the available literature as cited in the Appendix; however, Direct Brown 95 does not contain functional groups that are readily susceptible to environmental hydrolysis. Therefore, hydrolysis may not be environmentally significant.

2.1.2. Oxidation/Reduction. Specific experimental data regarding the oxidation/reduction of Direct Brown 95 could not be located in the available literature as cited in the Appendix. Takemura et al. (1965) reported that bubbling H_2S through a pure azo-dye solution yields aromatic amines and suggested that azo dyes in wastewater may be reduced at the azo linkage (by H_2S or SO_2 in the water) to form compounds such as benzidine or naphthylamine, from which the dye was produced; however, a water system receiving dyes would have to be very polluted so that H_2S or SO_2 (or a reducing environment) would significantly convert the dye to its corresponding parent amine.

2.1.3. Photolysis. Porter (1973) exposed an aqueous solution of Direct Brown 95 to a carbon arc light source and measured the degradation rate. The solution was contained in a pyrex cell having a limiting absorbance of 1.0 at 280 nm and the temperature was maintained at an average of 50°C; ~40% of the Direct Brown 95 was photodegraded during 200 hours of irradiation. Comparison of the artificial light to natural sunlight using Direct Blue 76 and Basic Green 4 showed that these two dyes degraded at least 10 times slower in natural sunlight. The author concluded that direct dyes are relatively stable to direct photolysis in natural waters.

Haag and Mill (1985) examined the aqueous photodegradation rate of 15 azo dyes by simulated sunlight or natural sunlight. Direct photolysis was observed for some dyes; however, humic materials in natural water were found to accelerate the photodecomposition rate of the dyes indirectly. Direct Brown 95 was not one of the dyes used in this study. Since the indirect photooxidation by singlet oxygen was found to be significant for dyes that existed in anionic form in natural water, this process may be significant with Direct Brown 95 if it exists in anionic form in natural waters.

2.1.4. Microbial Degradation. A COD, 5-day BOD and 20-day BOD of 640,500, 19,400 and 15,400, respectively, have been reported for Direct Brown 95 by a former manufacturer (Atlantic Chemical Co., 1982), which indicates relatively poor biodegradation of this dye.

Brown et al. (1981) measured the aerobic respiration rate of activated sludge in the presence of Direct Brown 95. At a concentration of 100 mg/ℓ, a small inhibitory effect was observed, which declined with time over a 24-hour period. At a concentration of 10 mg/ℓ, a slight inhibitory effect was observed after 0.5 and 3 hours of incubation, while a slight stimulation in respiration occurred after 24 hours. According to the authors, the results indicate that Direct Brown 95 is unlikely to be significantly toxic to aerobic sewage treatment at low concentrations.

Yoshida et al. (1971b) found that the benzidine-derived dye, Direct Black 38, was reduced by natural soil inocula to the corresponding benzidine derivative; however, the benzidine derivative was found to be stable towards soil bacteria, and no further change was observed after 9 weeks

Although Direct Brown 95 was not one of the dyes tested, Brown and Laboureur (1983) reported that a number of azo dyes were substantially biodegraded under anaerobic test conditions and suggested that the breakdown of dyestuffs in the environment may be initiated under anaerobic conditions.

2.1.5. Volatilization. Experimental volatilization data could not be located in the available literature as cited in the Appendix; however, since Direct Brown 95 is soluble in water (Society of Dyers and Colourists, 1971) and may have a relatively low vapor pressure because of its ability to form agglomerates through hydrogen binding (see Section 1.2.), a relatively small Henry's Law constant is expected. Therefore, volatilization from water is not expected to be significant in the environment.

2.1.6. Adsorption. Experimental adsorption data specific to Direct Brown 95 could not be located in the available literature as cited in the Appendix. Dyestuffs by their substantive nature (ability to be exhaustively deposited to fibers from aqueous baths) are likely to be adsorbed onto both sewage works sludge and silts and sediments of rivers and lakes (Brown and Laboureur, 1983). In conventional biological waste treatment systems, soluble dye removal usually occurs when the dye is adsorbed onto sludge (Porter, 1973). Sewage works sludge is usually treated by anaerobic digestion or landfilling. Removal of the dye from the sludge must, therefore, occur by anaerobic biodegradation. Similar anaerobic conditions exist in many lakes and rivers with respect to adsorbed material on silts and sediments and, therefore, adsorbed dyes may be subject to anaerobic biodegradation in the environment (Brown and Laboureur, 1983).

2.1.7. Bioconcentration. Experimental bioconcentration data could not be located in the available literature as cited in the Appendix. Since Direct Brown 95 is water soluble (Society of Dyers and Colourists, 1971), bioconcentration in aquatic organisms is not expected to be significant.

2.2. AIR

Direct Brown 95 in the atmosphere is most likely to be associated with particulate matter and dusts, especially from atmospheric effluents resulting from production and use. These particulate matter and dusts are subject to wet and dry deposition.

2.3. SOIL

Pertinent experimental data regarding the fate of Direct Brown 95 in soil could not be located in the available literature as cited in the Appendix. Chemical degradation may not significantly occur in soil since dyes in general are resistant to oxidative degradation (Porter, 1973) and hydrolysis is not expected to be environmentally significant. If significant transformation of Direct Brown 95 is to occur in soil, microbial degradation may be the environmental fate process involved. A number of azo dyes have been shown to be susceptible to anaerobic biodegradation (Brown and Laboureur, 1983) and the benzidine-derived dye Direct Black 38 has been shown to be reduced to the parent benzidine with soil inocula (Yoshida et al., 1971b). Photodegradation of Direct Brown 95 beyond the surface layers may not be important.

Since Direct Brown 95 is water soluble (Society of Dyers and Colourists, 1971), leaching in soil might be expected. The substantive nature of dyestuffs (Brown and Laboureur, 1983), including the direct dyes (Kuehni et al., 1979), indicates, however, that significant adsorption to soil may occur. Therefore, significant leaching in soil would not occur.

2.4. SUMMARY

Very little experimental data specific to Direct Brown 95 are available to predict its environmental fate. Porter (1973) concluded that Direct Brown 95 is relatively stable to direct photolysis in natural waters.

Biodegradation studies with Direct Brown 95 have indicated relatively poor biodegradation based on 5- and 20-day BODs (Atlantic Chemical Co., 1982). If released to water, Direct Brown 95 may be susceptible to significant adsorption because dyestuffs by their substantive nature (ability to be exhaustively deposited to fibers from aqueous baths) are likely to be adsorbed onto both sewage works sludge and silts and sediments of rivers and lakes (Brown and Laboureur, 1983). Removal of adsorbed Direct Brown 95 may occur by anaerobic biodegradation, since a number of azo dyes have been found to be substantially biodegraded under anaerobic conditions (Brown and Laboureur, 1983). Hydrolysis, volatilization and bioconcentration are not expected to be significant; adsorption and microbial degradation are expected to be the important fate processes in water. If released to the atmosphere, Direct Brown 95 will likely be associated with particulate matter and dusts that are subject to wet and dry deposition. If released to soil, Direct Brown 95 may not be subject to significant leaching since dyestuffs are susceptible to significant adsorption. Reductive degradation within soil may occur by anaerobic microbial processes (Yoshida et al., 1971b). Photodegradation beyond soil surfaces is not expected to be important.

3. EXPOSURE

Direct Brown 95 does not occur in nature (IARC, 1982a); therefore, exposure from natural sources is not expected to occur. Occupational exposure to Direct Brown 95 occurs during its production and use for the dyeing of textiles, leather and paper (IARC, 1982a). Likely routes of exposure are inhalation of particulates and dermal exposure. A National Occupational Hazard Survey, conducted between 1972 and 1974, estimated that 1113 U.S. workers are potentially exposed to Direct Brown 95 (NIOSH, 1984); a later survey conducted by NIOSH estimated that ~700 workers are exposed (IARC, 1982a).

3.1. WATER

Pertinent data could not be located in the available literature as cited in the Appendix. For the organic dye industry in general, it has been estimated that ~10% of the dye used for fabric is lost to wastewater effluents during dye operations (Porter, 1973; Brown et al., 1981). Losses of dyes to wastewater effluents during manufacture have been estimated to be 1-2% (Brown et al., 1981). Waste streams from dye manufacture contain dissolved inorganic salts and small amounts of dye and dye intermediates (Steadman et al., 1977). Wastewater effluents are therefore the major source of release to the aquatic environment. The efficiency of wastewater treatment operations will determine the amount of dye which reaches natural waters.

Release of dye from textile fabrics may occur from commercial or consumer washing of the fabric; however, dyes in most finished products are considered to be essentailly "fast" (they do not migrate or wash out) (Jones, 1979). Various after-treatments are commonly applied to fabrics after dyeing with direct dyes to improve their color fastness (Kuehni et al., 1979).

3.2. FOOD

Pertinent data regarding exposure to Direct Brown 95 through food could not be located in the available literature as cited in the Appendix.

3.3. INHALATION

Total particulate levels of Direct Brown 95 detected in workplace air were 1.3-1.54 mg/m^3 at a textile-dyeing operation; 1.12-14.72 mg/m^3 at a leather-dyeing operation; and 0.17-3.30 mg/m^3 at a paper-dyeing operation (IARC, 1982a). No ambient air monitoring data were located.

Ambient atmospheric emissions may occur from ventilation of effluents at production and use sites. In addition, air effluents from production operations such as spray drying may be sources of release. Particulate emissions from these sources are usually filtered at the plant site, which should significantly reduce the amount of particulate matter actually reaching the ambient atmosphere.

3.4. DERMAL

Other than possible dermal exposure during production and use for commercial dyeing, pertinent monitoring data could not be located in the available literature as cited in the Appendix. The general public is exposed mainly to finished dyes after they have been applied to the product; however, there is little chance of dyes coming off in perspiration, saliva, or washings if label instructions are followed (Jones, 1979).

3.5. SUMMARY

Occupational exposure to Direct Brown 95 occurs during production and use in dyeing (IARC, 1982a). Wastewater effluents are probably the major source of release to the aquatic environment. Ambient monitoring data could not be located in the available literature as cited in the Appendix.

4. PHARMACOKINETICS

4.1. ABSORPTION

Pertinent data regarding the absorption of Direct Brown 95 could not be located in the available literature as cited in the Appendix.

4.2. DISTRIBUTION

Pertinent data regarding the distribution of Direct Brown 95 could not be located in the available literature as cited in the Appendix.

4.3. METABOLISM

NIOSH (1980a) and Lowry et al. (1980) collected environmental samples and urine samples from workers occupationally exposed to benzidine-based dyes, including Direct Brown 95. Levels of 3 and 7 ppb monoacetylbenzidine were detected in 2/8 workers at one plant. At another facility, four workers exposed to average levels of 4.3, 5.2, 11.7 and 17.4 mg total particulate/m^3 of benzidine-based dyes had urinary concentrations of 52, 11, 10 and 112 ppb benzidine. Urine samples containing 112, 52 and 11 ppb benzidine had concentrations of 590, 248 and 22 ppb monoacetylbenzidine present, respectively. U.S. EPA (1980) considered these findings especially alarming because these levels of benzidine were greater than those measured by Zavon et al. (1973) in benzidine-exposed workers with bladder cancer. Samples of urine taken from workers at other facilities contained no benzidine. Metabolite levels were well in excess of those expected for the impurities present (NIOSH, 1980a).

Walker (1970) suggested that benzidine-based dyes may be degraded in the gut and liver to free benzidine or monoacetylbenzidine.

Rinde and Troll (1975) gave Direct Brown 95 to rhesus monkeys by stomach tube at two different doses equivalent to 7 and 35 mg of benzidine. Urine collected for 72 hours was examined for metabolites, and both benzidine and

monoacetylbenzidine were found. The total amount of benzidine plus monoacetylbenzidine was 0.10 mg after the 7 mg dose and 0.41 mg after the 35 mg dose. These quantities represented 1.5 and 1.2%, respectively, of the benzidine moiety in Direct Brown 95.

In a 13-week toxicity study, NCI (1978) fed male and female B6C3F1 mice up to 12,000 ppm Direct Brown 95 in their diets, while male and female Fischer 344 rats received up to 3000 ppm in their diets. Benzidine and monoacetylbenzidine were found in urine collected over a 24-hour period from both sexes of both species.

4.4. EXCRETION

Data regarding the amount and rate of excretion of Direct Brown 95 could not be located in the available literature, but the metabolites, benzidine and monoacetylbenzidine, are excreted in the urine following occupational exposure of humans to benzidine-based dyes (NIOSH, 1980a) and oral exposure of rats, mice (NCI, 1978) and monkeys (Rinde and Troll, 1975) to Direct Brown 95.

4.5. SUMMARY

Pertinent data regarding the absorption and distribution of Direct Brown 95 could not be located in the available literature as cited in the Appendix. Walker (1970) suggested that benzidine-based dyes may be degraded in the gut and liver to free benzidine and monoacetylbenzidine. Benzidine and monoacetylbenzidine were detected in the urine of workers occupationally exposed to benzidine-based dyes (NIOSH, 1980a), and in the urine of rats, mice and monkeys orally-administered Direct Brown 95 (NCI, 1978; Rinde and Troll, 1975).

5. EFFECTS

5.1. CARCINOGENICITY

5.1.1. Animal Studies. NCI (1978) gave groups of 10 male and 10 female Fischer 344 rats and 10 male and 10 female B6C3F1 mice Direct Brown 95 (~72% pure with no benzidine contamination; detection limit 0.004% by HPLC) in corn oil in the diet for 91 days with 2 additional observation days. Rats were exposed to concentrations of 190, 375, 750, 1500 and 3000 ppm. Male mice were given concentrations of 750, 1500, 3000, 6000 and 12,500 ppm and female mice were exposed to 375, 750, 1500, 3000 and 6000 ppm. Control groups of 10 rats and 10 mice of each sex were given corn oil only in the diet. A dose-related increase in mortality was observed in rats, but not in mice. All rats of both sexes given 1500 and 3000 ppm Direct Brown 95 and two males given 750 ppm died before the end of the study. Mortality was unaffected in the other dosage groups and in controls. Survival was unaffected in mice. All animals that died, that were killed in a moribund condition or that were killed at the end of the study were necropsied. Unless precluded by cannibalism or autolysis, microscopic examination of skin, lungs, bone marrow, spleen, mandibular and mesenteric lymph nodes, thymus, heart, salivary gland, liver, bile duct (mice), pancreas, stomach, small intestine, colon, kidney, bladder, adrenals, thyroids, testes and epididymis was performed on controls, rats treated at $\geq$750 ppm, male mice treated at 12,500 ppm and female mice treated at 6000 ppm. These tissues were also examined in one male mouse at 750 ppm that died early. Microscopic examination of all other groups of rats was limited to the liver, spleen, bone marrow, kidney and pancreas. Microscopic examination of other groups of mice was limited to the liver and kidney of males at $\geq$3000 ppm and females at $\geq$1500 ppm and the spleens of all mice. Liver tumors were

observed in female rats at 1500 ppm (Table 5-1), but not in male rats or in controls. The time to observation of the first tumor was 5 weeks as compared with ~8 weeks in the historic controls. The absence of liver tumors at the highest dose may be attributed to the early deaths of these rats. A statistically significant ($p \leq 0.001$) increased incidence in preneoplastic hepatic lesions (basophilic foci) was observed in male rats (7/8) given 1500 ppm Direct Brown 95. Lower incidences of basophilic foci were observed in male rats given 750 (3/10) or 3000 (2/9) ppm Direct Brown 95 and in female rats given 1500 (3/8) or 3000 (3/8) ppm. Statistically significant results were obtained at lower doses when foci of cellular alteration were added to the basophilic foci. The incidences of basophilic foci or foci of cellular alterations were 10/10 males at 375 ppm ($p<0.001$), 5/10 males at 750 ppm ($p=0.016$), 7/8 males at 1500 ppm ($p<0.001$), 2/9 males at 3000 ppm (NS), 3/10 females at 375 ppm and at 750 ppm (NS), 6/8 females at 1500 ppm ($p=0.002$) and 3/8 females at 3000 ppm (NS). No tumors were observed in mice. One mouse at 12,500 ppm exhibited hepatocellular basophilic foci, which may indicate the possible onset of neoplastic lesions; however, the mice were tested for only 13 weeks and there may not have been sufficient time for tumors to develop. NCI (1978) concluded that Direct Brown 95 was carcinogenic in female rats. IARC (1982a) considered this evidence to be limited.

5.1.2. Human Studies. Human studies for Direct Brown 95 alone could not be located in the available literature as cited in the Appendix. IARC (1982b,c) summarized the numerous case studies reporting the occurrence of bladder tumors in dyestuff workers, as well as several epidemiological studies suggesting excess mortality from bladder cancer in people occupationally exposed to benzidine-based dyes. Interpretation of these studies is difficult because workers were exposed to mixtures of several dyes in

TABLE 5-1

Incidence of Liver Tumors in Female Fischer 344 Rats Given 190-3000 ppm of Direct Brown 95 (no benzidine contamination) in the Diet for 91 Days and Observed for an Additional 2 Days[a]

Dose (ppm)	Tumor Incidence (p value)
0	0/10
190	0/10
375	0/10
750	0/10
1500	5/8[b] (p=0.007)
3000	0/8

QUALITY OF EVIDENCE

Strengths of Study: Both sexes of two animal species (rats and mice) were tested by a relevant route of administration (dietary) at several concentrations. The MTD was estimated by pretesting. The data were analyzed statistically. Histopathological examination was comprehensive at the higher doses and limited at lower doses.

Weakness of Study: The test did not last for the lifespan of the animals tested.

Overall Adequacy: Sufficient

[a]Source: NCI, 1978

[b]One rat had a hepatocellular carcinoma and four rats had neoplastic nodules.

these occupational settings. IARC (1982b) reviewed and evaluated a Japanese hospital-based, case-control study of 200 cases of bladder cancer in men and 148 male controls of the same age with urinary disorders (Yoshida and Miyakawa, 1972; Yoshida et al., 1971a). Seventeen (8.5%) of the cancer cases and 2 (1.4%) of the controls worked in the silk-dyeing industry. Employment in the silk-dyeing industry had a relative risk of 6.8 ($p=0.002$). Seven of the 17 bladder cases were painters who ingested the benzidine-based dyes through frequent oral contact with their painting tools. IARC (1982a,b,c,d) concluded that exposure to benzidine-based dyes was strongly associated with the occurrence of bladder cancer in humans. Although the epidemiological data were inadequate for evaluating the possible carcinogenic effects of Direct Brown 95 alone on man, when considered together with the fact that benzidine was present in the urine of exposed workers provided "sufficient evidence that occupational exposure to benzidine-based dyes represent a carcinogenic risk to man."

5.2. MUTAGENICITY

Direct Brown 95 has been tested for mutagenicity and genotoxicity, and the details are summarized in Table 5-2. Prival et al. (1984) found that in the presence of uninduced hamster liver S-9 Direct Brown 95 was mutagenic in Salmonella typhimurium TA98 without FMN at 0.1 and 0.3 µmol/plate; increased mutagenic activity was observed at 0.01 and 0.03 µmol/plate when FMN was added to the test system. Joachim et al. (1985) found that Direct Brown 95 was not mutagenic in five strains of S. typhimurium in the presence of Aroclor-induced rat liver S-9, but when the dye was reduced by dithionite, mutagenic activity occurred in TA98 and TA1538. Reid et al. (1984) studied the mutagenic activity of Direct Brown 95 in TA1538 in the presence of S-9 before and/or after bacterial (or FMN) reduction; bacterial reduction enhanced the mutagenic activity of Direct Brown 95 in S. typhimurium TA1538.

TABLE 5-2

Mutagenicity Testing of Direct Brown 95

Assay	Indicator/ Organism	Application	Concentration or Dose	Activating System	Response	Comments	Reference
Reverse mutation	Salmonella typhimurium TA98	plate incorporation	0.01-0.03 µmol/plate	+S-9 ±FMN	‡	Positive response without FMN at higher doses 0.1 and 0.3 µmol/plate; FMN increased mutagenic activity	Prival et al., 1984
	S. typhimurium TA1535, TA1537, TA1538, TA98, TA100	plate incorporation	≤5 mg/plate	+S-9	-	NC	Joachim et al., 1985
	S. typhimurium TA1535 TA1537 TA1538 TA98 TA100	plate incorporation	≤5 mg/plate	+S-9 and dithionite	- - + + -	Dithionite chemically reduces the azo bond in the dye	Joachim et al., 1985
	S. typhimurium TA1538	plate incorporation	0.25-1.0 µmol/plate	+S-9 rat, ± bacterial reduction	±	Bacterial reduction enhanced mutagenic activity	Reid et al., 1984
	S. typhimurium TA1538	plate incorporation	100-1000 µg/plate	±S-9	=	NC	Joachim and Decad, 1984
UDS	male Sprague-Dawley rat hepatocytes	gavage	100 mg/kg	NA	+	NC	Joachim and Decad, 1984
	rat hepatocytes	in vitro	50-500 mg/mL	NA	-	NC	Joachim and Decad, 1984

NC = No comment; NA = not applicable

Joachim and Decad (1984) found that *in vitro* Direct Brown 95 did not cause an increase in UDS in rat hepatocytes. However, when the compound was given to rats by gavage at 100 mg/kg, a significant increase in the amount of UDS was observed in hepatocytes isolated 17 hours after treatment. These results indicate that microsomal metabolism alone was insufficient to produce a mutagenic response and that reduction of the azo bond was required.

5.3. TERATOGENICITY

Pertinent data regarding the teratogenicity of Direct Brown 95 could not be located in the available literature as cited in the Appendix.

5.4. OTHER REPRODUCTIVE EFFECTS

Pertinent data regarding the reproductive effects of Direct Brown 95 could not be located in the available literature as cited in the Appendix.

5.5. CHRONIC AND SUBCHRONIC TOXICITY

NCI (1978) gave groups of 10 Fischer 344 rats and 10 B6C3F1 mice of each sex Direct Brown 95 in the diet for 91 days with 2 additional observation days. The rats were exposed to concentrations of 190, 375, 750, 1500 and 3000 ppm. Male mice were exposed to concentrations of 750, 1500, 3000, 6000 and 12,500 ppm and female mice were exposed to 375, 750, 1500, 3000 and 6000 ppm (see Section 5.1.). A dose-related decrease in mean body weight gain occurred in male and female rats at $\geq$375 ppm. Body weight gain of mice was lower than control at 6000 and 12,500 ppm in males and at 6000 ppm in females. There was a dose-related increase in mortality in male and female rats; all rats at 1500 and 3000 ppm and two male rats at 750 ppm died before the end of the study. Mortality was not affected in mice. Necropsies and microscopic examinations were performed as described in Section 5.1. Several general trends were reported for liver lesions in rats; these trends revealed a progression of change from a normal to preneoplastic condition to

neoplasms. Rats that survived for longer periods of time had more proliferative liver changes as well as preneoplastic and neoplastic lesions than rats that were moribund earlier. Liver changes were of two types, either hyperplasia with multiple foci of cellular alteration and nodules, or cirrhosis with nodules. The first type of liver changes were considered preneoplastic and neoplastic (see Section 5.1.). Cirrhosis (nodular regeneration and biliary hyperplasia, focal and biliary hyperplasia), which occurred along with neoplastic nodules and carcinomas, can be considered nonneoplastic lesions. Biliary hyperplasia, nodular regeneration and portal fibrosis were observed in male rats exposed to 750 ppm Direct Brown 95. Female rats exhibited nodular regeneration and portal fibrosis at 750 ppm, but not biliary hyperplasia. These cirrhotic changes occurred in the longer-lived rats. More rapidly occurring nonneoplastic effects of Direct Brown 95 included lymphoid depletion of the spleen and thymus, myeloid depletion of the bone marrow and varying degrees of biliary hyperplasia. These effects were noted in the first rats that died. Male rats exhibited lymphoid depletion of the spleen and thymus at $\geq$750 ppm and myeloid depletion of the bone marrow at $\geq$1500 ppm. Female rats had lymphoid depletion of the spleen and myeloid depletion of the bone marrow at $\geq$750 ppm as well as lymphoid depletion of the thymus at $\geq$1500 ppm. Glomerulonephritis was observed at 750 ppm in both sexes of rats that survived to the end of the experiment. Female rats given 375 ppm Direct Brown 95 exhibited degenerative changes in pancreatic acinar epithelial cells, but these lesions did not occur in these groups. None of these lesions were found in controls.

Male mice exposed to concentrations $\geq$6000 ppm exhibited hemosiderosis of the kidney and pigment deposition in the liver. Hemosiderosis of the spleen was observed at $\geq$750 ppm in male mice. Female mice displayed pigment

deposits in the liver at concentrations ≥3000 ppm, hemosiderosis of the spleen at concentrations of ≥375 ppm and hemosiderosis of the kidney at 6000 ppm. Hepatocellular degeneration was observed in 10/10 male mice exposed to 12,500 ppm Direct Brown 95; one male mouse at this concentration also exhibited foci of cellular alteration of the liver (hepatocytes were distinctly basophilic). These lesions were not observed in other treated groups or in controls.

5.6. OTHER RELEVANT INFORMATION

Oral LD_{50} values for Direct Brown 95 could not be located in the available literature as cited in the Appendix. In a 2-week range-finding study, groups of five male and five female Fischer 344 rats and five male and five female B6C3F1 mice were treated with Direct Brown 95 in the diet at 3000, 6000, 12,500, 25,000 or 50,000 ppm (NCI, 1978). Dose-related, severely decreased food consumption and mean body weight gain occurred at ≥3000 ppm for rats. One 50,000 ppm male rat and all 12,500 female rats died within 11-12 days. All other rats survived. Gross necropsy revealed thymic atrophy (from low food consumption), splenic enlargement and dose-related darkening of the spleen and kidney at unspecified doses. In mice, no deaths occurred. Dose-related decreased mean body weight gain occurred at ≥6000 ppm in both sexes. Hunched appearance and lethargy occurred at the higher concentrations (not otherwise specified). No toxicologically significant gross lesions were observed.

5.7. SUMMARY

Direct Brown 95 was carcinogenic in female rats, inducing liver tumors within only 13 weeks of dietary administration (NCI, 1978). No neoplastic effect was observed in male rats or in mice of either sex in the 13-week study. Case reports (IARC, 1982b,c) and epidemiological studies (Yoshida and Miyakawa, 1972; Yoshida et al., 1971a) associating occupational exposure

to benzidine-based dyes with bladder cancer and the finding of benzidine in the urine of workers exposed to the dyes (NIOSH, 1980a) were sufficient to conclude that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man (IARC, 1982a,b,c,d). The data were inadequate, however, to assess the carcinogenic risk of occupational exposure to Direct Brown 95 alone.

Direct Brown 95 was generally not mutagenic to S. typhimurium in the presence of S-9 unless the diazo bond is reduced either by a reducing agent or cecal bacteria (Prival et al., 1984; Reid et al., 1984; Joachim et al., 1985). Direct Brown 95 induced UDS in rat hepatocytes when administered in vivo but not in vitro (Joachim and Decad, 1984). Pertinent data regarding teratogenicity or reproductive effects were not available.

In the 13-week dietary study by NCI (1978), dose-related decreased body weight gain occurred in rats at ≥375 ppm and at ≥6000 ppm in mice. Dose-related increased mortality occurred in rats at ≥750 ppm. Nonneoplastic lesions in rats included cirrhotic liver changes, lymphoid depletion of the spleen and thymus, myeloid depletion of the bone marrow, biliary hyperplasia and glomerulonephritis at ≥750 ppm. No effects were observed in rats at 190 ppm. Mice had hemosiderosis of the spleen at ≥350 ppm (lowest dose tested), pigment deposition in the liver at ≥3000 ppm, hemosiderosis of the kidney at ≥6000 ppm and hepatocellular degeneration at 12,500 ppm. A consideration of metabolism and related factors is important in that formulations of Direct Brown 95 apparently have varying amounts of residual benzidine as a contaminant and that mammals including monkeys have been shown to metabolize Direct Brown 95 to benzidine and acetalbenzidine to some degree. The presence of benzidine in the urine of workers exposed to benzidine-based dyes together with significant incidence of bladder cancer demonstrates an important correlation.

6. AQUATIC TOXICITY

6.1. ACUTE

Little et al. (1974) reported the 96-hour LC_{50} of Direct Brown 95 to fathead minnows, Pimephales promelas, to be >180 mg/ℓ. This value is substantially higher than the concentrations that produce color (0.4-1.1 mg/ℓ) (Little et al., 1974). No other data concerning the aquatic toxicity of this compound could be located in the available literature as cited in the Appendix.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

OSHA/NIOSH (1980) and NIOSH (1980b, 1983) considered the benzidine-based dyes to be potential human carcinogens. OSHA/NIOSH (1980) recommended that occupational exposure be reduced to the lowest feasible level. In response to ITC recommendations, U.S. EPA (1981) chose not to require additional testing of benzidine, o-toluidine and o-dianisidine based dyes for further health and environmental effects.

7.2. AQUATIC

Guidelines and standards for the protection of aquatic biota from the effects of Direct Brown 95 could not be located in the available literature as cited in the Appendix.

8. RISK ASSESSMENT

Direct Brown 95 was carcinogenic in female rats, inducing liver tumors within only 13 weeks of dietary administration (NCI, 1978). No neoplastic effect was observed in male rats or in mice of either sex in the 13-week study. Case reports (IARC, 1982b,c) and epidemiological studies (Yoshida and Miyakawa, 1972; Yoshida et al., 1971a) associating occupational exposure to benzidine-based dyes with bladder cancer and the finding of benzidine in the urine of workers exposed to the dyes (NIOSH, 1980a) were sufficient to conclude that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man (IARC, 1982a,b,c,d). The data were inadequate, however, to assess the carcinogenic risk of occupational exposure to Direct Brown 95 alone.

Direct Brown 95 was generally not mutagenic to S. typhimurium in the presence of S-9 unless the diazo bond is reduced either by a reducing agent or cecal bacteria (Prival et al., 1984; Reid et al., 1984; Joachim et al., 1985). Direct Brown 95 induced UDS in rat hepatocytes when administered in vivo but not in vitro (Joachim and Decad, 1984). Pertinent data regarding teratogenicity or reproductive effects were not available.

In the 13-week dietary study by NCI (1978), dose-related decreased body weight gain occurred in rats at ≥375 ppm and at ≥6000 ppm in mice. Dose-related increased mortality occurred in rats at ≥750 ppm. Nonneoplastic lesions in rats included cirrhotic liver changes, lymphoid depletion of the spleen and thymus, myeloid depletion of the bone marrow, biliary hyperplasia and glomerulonephritis at ≥750 ppm. No effects were observed in rats at 190 ppm. Mice had hemosiderosis of the spleen at ≥350 ppm (lowest dose tested), pigment deposition in the liver at ≥3000 ppm, hemosiderosis of the kidney at ≥6000 ppm and hepatocellular degeneration at 12,500 ppm.

NCI (1978) concluded that Direct Brown 95 was carcinogenic in rats by the oral route and IARC (1982a) considered the evidence to be limited. Therefore, it is appropriate to derive a q_1^*. Data used to calculate the q_1^* are presented in Table 8-1.

The transformed animal doses were calculated by multiplying the dietary concentrations by a food factor of 0.05, by 91 days/93 days and by the cube root of the ratio of the rat body weight to the reference human body weight (70 kg). The body weight ratio correction was made before calculating the q_1^* because the average weights varied substantially among the groups (see Table 8-1). The unadjusted q_1^* was calculated using the computerized multistage model developed by Howe and Crump (1982). When the high-dose data was included, the dose-response data did not fit the model; therefore, the high dose was dropped. The absence of tumors in the high-dose group was a consequence of the early deaths of rats in this group. The unadjusted q_1^* of 1.92×10^{-2} $(mg/kg/day)^{-1}$ was multiplied by the cube of the ratio of the lifespan of rats (730 days) to the length of the experiment (93 days) to yield the human q_1^* of 9.3 $(mg/kg/day)^{-1}$. The water concentration associated with an increased lifetime risk of cancer at levels of 10^{-5}, 10^{-6} and 10^{-7} are 3.8×10^{-5}, 3.8×10^{-6} and 3.8×10^{-7} mg/ℓ, assuming a 70 kg man consumes 2 ℓ water/day.

TABLE 8-1

Cancer Data Sheet for Derivation of q_1^* for Direct Brown 95

Compound: Direct Brown 95

Reference: NCI, 1978

Species/strain/sex: rat/Fischer 344/female

Route/vehicle: oral/diet

Length of exposure (le) = 91 days

Length of experiment (Le) = 93 days

Lifespan of animal (L) = 730 days

Body weight = 0.150 kg (control and 190 ppm); 0.140 kg (375 ppm); 0.120 kg (750 ppm); 0.085 kg (1500 ppm); and 0.075 kg (3000 ppm)

Tumor site and type: liver tumors (hepatocellular carcinoma and neoplastic nodules)

Experimental Doses or Exposures* (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested
0	0	0/10
190	1.2	0/10
375	2.3	0/10
750	4.4	0/10
1500	7.8	5/8
3000	15.0	0/8

Unadjusted $q_1^* = 1.9196732 \times 10^{-2}$ $(mg/kg/day)^{-1}$

Human $q_1^* = 9.3$ $(mg/kg/day)^{-1}$

*The high dose was eliminated because the data did not fit the model when it was included.

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

The NCI (1978) bioassay reported several toxic effects associated with exposure to Direct Brown 95 (Table 9-1). The most severe effect was the increased mortality, which occurred at an equivalent human dose as low as 4.8 mg/kg/day. Multiplying 4.8 mg/kg/day by 70 kg and dividing by an uncertainty factor of 10 to approximate chronic exposure results in an MED of 33.6 mg/day, which corresponds to an RV_d of 3.2 (Table 9-2). Increased mortality warrants an RV_e of 10. Multiplying the RV_d by the RV_e yields a CS of 32, which corresponds to an RQ of 100.

The next most severe effect was cirrhosis of the liver, which occurred at an equivalent human dose as low as 4.5 mg/kg/day, which when multiplied by 70 kg and divided by an uncertainty factor of 10 yields an MED of 31.5 mg/day. The RV_d of 3.3 and an RV_e of 7 is warranted. Multiplying the RV_d by the RV_e results in a CS of 23, which corresponds to an RQ of 100.

Hemosiderosis of the spleen occurred at 3.1 mg/kg/day. Multiplying by 70 kg and dividing by 10 yields an MED of 21.7 mg/day, which corresponds to an RV_d of 3.5. Since hemosiderosis may be indicative of hemolytic anemia, an RV_e of 6 is warranted. The CS is 21, which corresponds to an RQ of 100.

Decreased body weight gain occurred at an equivalent human dose of 2.4 mg/kg/day or an MED of 16.8 mg/day. The RV_d is 3.7 and the RV_e is 4, which when multiplied together yields a CS of 15. This CS corresponds to an RQ of 1000.

Several other effects were observed, but these were less severe or occurred at higher doses; therefore, CSs need not be calculated for these effects.

TABLE 9-1

Oral Toxicity Summary for Direct Brown 95 (72% purity) Administered in the Diet[a,b]

Species/Strain	Sex	No. at Start	Average Weight[c] (kg)	Exposure (ppm)	Transformed Animal Dose[d] (mg/kg/day)	Equivalent Human Dose[e] (mg/kg/day)	Response
Rat/F344	M,F	10/sex	0.225 (M), 0.145 (F)	375 for 91 days	18.75	2.8 (M) 2.4 (F)	decreased body weight gain
	M,F	10/sex	0.15 (M), 0.12 (F)	≥750 for 91 days	37.5	4.8 (M) 4.5 (F)	increased mortality of males; cirrhosis of liver, lymphoid depletion of the spleen and thymus, myeloid depletion of bone marrow, glomerulonephritis both sexes
	M,F	10/sex	0.11 (M) 0.085 (F)	≥1500	75	8.7 (M) 8.0 (F)	100% mortality both sexes
Mouse/B6C3F1	F	10	0.018	375	48.75	3.1	hemosiderosis of spleen
	M	10	0.024	750	97.5	6.8	hemosiderosis of spleen
	F	10	0.017	3000	390	24.3	pigment deposits in liver, decreased body weight gain
	F	10	0.016	6000	780	47.7	hemosiderosis of the kidneys; decreased body weight gain
	M	10	0.020	6000	780	51.4	hemosiderosis of kidney, pigment deposits in liver, decreased body weight gain
	M	10	0.020	12,000	1560	102.7	hepatocellular degeneration

[a]Source: NCI, 1978

[b]The dye was free of benzidine

[c]Estimated from growth curves

[d]Calculated by multiplying the concentration by 0.05 for rats and 0.13 for mice

[e]Calculated by multiplying the transformed animal dose by the cube root of the ratio of the animal body weight to the reference human body weight (70 kg)

TABLE 9-2

Oral Composite Scores for Direct Brown 95[a]

Species	Dose/Exposure	Chronic Human MED[b] (mg/day)	RV_d	Effect	RV_e	CS	RQ
Rat	750 ppm in diet for 91 days	33.6	3.2	increased mortality	10	32	100
	750 ppm in diet for 91 days	31.5	3.3	liver cirrhosis	7	23	100
	375 ppm in diet for 91 days	16.8	3.7	decreased body weight gain	4	15	1000
Mouse	375 ppm in diet for 91 days	21.7	3.5	hemosiderosis of the spleen	6	21	100

[a]Source: NCI, 1978

[b]The doses were divided by an uncertainty factor of 10 to approximate chronic exposure.

Examination of Table 9-2 reveals that the highest CS of 32 was obtained from the data for increased mortality in rats. Therefore, these data are recommended as the basis for the RQ for Direct Brown 95 (Table 9-3).

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

Data regarding the carcinogenicity of Direct Brown 95 were discussed in Section 5.1. Direct Brown 95 was carcinogenic to female rats, inducing liver tumors within only 13 weeks of dietary administration (see Table 5-1) (NCI, 1978). No evidence of carcinogenicity was found in male rats or in mice of either sex administered the dye in the diet for 13 weeks. Therefore, there is limited evidence that Direct Brown 95 is carcinogenic in animals (IARC, 1982a,d). Case reports (IARC, 1982b,c) and epidemiological studies (Yoshida and Miyakama, 1972; Yoshida et al., 1971a) associating occupational exposure to benzidine-based dyes with bladder cancer and the finding of benzidine in the urine of workers exposed to the dyes (NIOSH, 1980a) were sufficient to conclude that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man (IARC, 1982a,b,c,d). The data were inadequate, however, to assess the carcinogenic risk of occupational exposure to Direct Brown 95 alone. IARC (1982d) classified Direct Brown 95 as a Group 2B chemical. Since the data in animals are limited and in humans are inadequate, Direct Brown 95 could be classified as a U.S. EPA Group C chemical. However, a consideration of metabolism and related factors is important in that formulations of Direct Brown 95 apparently have varying amounts of residual benzidine as a contaminant and that mammals including monkeys have been shown to metabolize Direct Brown 95 to benzidine and acetalbenzidine to some degree. The presence of benzidine in the urine of workers exposed to benzidine-based dyes together with significant incidence of bladder cancer demonstrates an important correlation. The strong

TABLE 9-3

Direct Brown 95

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose*:	33.6 mg/day
Effect:	increased mortality
Reference:	NCI, 1978
RV_d:	3.2
RV_e:	10
Composite Score:	32
RQ:	100

*Equivalent human dose

association of exposure to benzidinebased dyes, in general, and bladder cancer is sufficient to classify Direct Brown 95 as a U.S. EPA Group A chemical (U.S. EPA, 1986b).

As discussed in Chapter 8, a q_1^* was calculated for Direct Brown 95 based on the data for liver tumors in female rats in the NCI (1978) study. The same data can be used to calculate the F factor (Table 9-4). The transformed doses were calculated as described in Chapter 8. The unadjusted $1/ED_{10}$ was calculated using the multistage model without the high-dose group data because the data did not fit the model when this group was included. The absence of tumors in the high-dose group was attributed to the early deaths in this group. The unadjusted $1/ED_{10}$ of 0.213 $(mg/kg/day)^{-1}$ was multiplied by the cube of the ratio of the lifespan of the rat (730 days) to the duration of the study (93 days) to yield the F factor of 103 $(mg/kg/day)^{-1}$. Because the F factor is >100, Direct Brown 95 is placed in Potency Group 1, which along with a U.S. EPA grouping of A, gives Direct Brown 95 a HIGH hazard ranking under CERCLA.

TABLE 9-4

Derivation of Potency Factor (F) for Direct Brown 95

Reference:	NCI, 1978
Exposure route:	oral
Species:	rats
Strain:	Fischer 344
Sex:	female
Vehicle or physical state:	oral, diet
Body weight:	0.150 kg (control and 190 ppm); 0.140 kg (375 ppm); 0.120 kg (750 ppm); 0.085 kg (1500 ppm); 0.075 kg (3000 ppm)
Duration of treatment:	91 days
Duration of study:	93 days
Lifespan of animal:	730 days
Target organ:	liver
Tumor type:	liver tumors (hepatocellular carcinoma and neoplastic nodules)
Experimental doses/Exposures (ppm):	0, 190, 375, 750, 1500
Transformed doses (mg/kg/day):	0, 1.2, 2.3, 4.4, 7.8, 15.0
Tumor incidence:	0/10, 0/10, 0/10, 0/10, 5/8, 0/8
Unadjusted $1/ED_{10}$:	0.2126931 $(mg/kg/day)^{-1}$
Adjusted $1/ED_{10}$ (F factor):	103 $(mg/kg/day)^{-1}$

Direct Brown

10. REFERENCES

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APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

GLOBAL
TSCATS
CASR online (U.S. EPA Chemical Activities Status Report)
CAS online STN International
TOXLINE
TOXBACK 76
TOXBACK 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register

These searches were conducted in May, 1985. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1986. Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 1985-1986. TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1985-1986. Cincinnati, OH. 114 p.

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ITII (International Technical Information Institute). 1982. Toxic and Hazardous Industrial Chemicals Safety Manual for Handling and Disposal with Toxicity and Hazard Data. ITII, Tokyo, Japan. 700 p.

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Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY.

Windholz, M., Ed. 1983. The Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ.

Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

Battelle's Columbus Laboratories. 1971. Water Quality Criteria Data Book. Volume 3. Effects of Chemicals on Aquatic Life. Selected Data from the Literature through 1968. Prepared for the U.S. EPA under Contract No. 68-01-0007. Washington, DC.

Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. U.S. Dept. Interior, Fish and Wildlife Serv. Res. Publ. 137, Washington, DC.

McKee, J.E. and H.W. Wolf. 1963. Water Quality Criteria, 2nd ed. Prepared for the Resources Agency of California, State Water Quality Control Board. Publ. No. 3-A.

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